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Diffusion Constant and Diffusion Coefficient

J. Verduin has pointed out that the Krogh diffusion constant has been erroneously regarded as an index of diffusivity of gases in liquid media (*1*). The confusion has arisen from failure by some biologists (i) to examine the units used by Krogh (*2*) and (ii) to compare them with those of the true diffusion coefficient. For his concentration gradient, Krogh has used the difference in partial pressure of gas per unit length of liquid phase, thereby introducing solubility into his diffusion constant; this distinguishes it from the diffusion coefficient.

I join Verduin in criticizing those biologists who have made gross errors in their use of the Krogh diffusion constant. However, a number of points in Verduin's paper may be misleading and are therefore discussed here.

1) It is worth noting that the value of D, the diffusion coefficient, for O_2 in water at 16°C (1.607 cm²/day) quoted by Spoehr (*3*) was not directly determined but was derived by Carlson (*4*) from his own experimental determination of 1.720 cm²/day at 18.2°C. Carlson assumed a 3-percent change in D per degree change in temperature to derive a value for 16°C.

Verduin has misquoted the value calculated by Carlson and quoted by Spoehr. He quotes it as 1.607 cm²/day at 20°C. As a consequence, Verduin's calculation of a Krogh diffusion constant at 20°C of 0.346 has no foundation.

2) The effect of temperature on the diffusivity of oxygen bears closer examination than that given by Verduin.

Table 1 summarizes, first, quoted values of D for O_2 in water (or dilute aqueous solutions) and second, values used for the increase in D with temperature for various substances in water.

Table 2 gives values for the increase in the diffusion coefficient of oxygen in water per degree rise in temperature. A linear increase per degree has been assumed and only the experimentally determined values of D from Table 1 have been used.

It appears from Table 2 that it is reasonable to suggest that the diffusion coefficient for oxygen in water increases linearly about 3 percent per degree rise in temperature within the range of 16° to 25°C.

The figure used by Verduin, 1.6 percent per degree Celsius (*15*), is a characteristic of the diffusion current measured in polarographic determinations and is dependent on a number of other variables in addition to the diffusion coefficient of the substance being examined.

3) Krogh asserts that his diffusion constant increased about 1 percent per degree rise in temperature. Verduin maintains that this statement is false and that there is no change in the Krogh constant within the temperature range of 20° to 30°C.

Table 1. Values of D and its change with temperature for O_2 in aqueous media

Temperature (°C)	Substance	Original measure of D	D (10^{-5} cm²/sec)	Approx. linear increase in D (%/°C rise in T)	Authority
16	O_2	1.62 cm²/day	1.875		Hüfner (*5*)
18	O_2		1.98	3*	Carlson (*6*)
18.2	O_2	1.720 cm²/day	1.99	3*	Carlson (*4*)
22	O_2	2.22×10^{-5} cm²/sec	2.22†	3*	Brdička and Wiesner (*7*)
25	O_2	2.38×10^{-5} cm²/sec	2.38	‡	Kolthoff and Laitinen (*8*)
25	O_2	2.6×10^{-5} cm²/sec	2.6		Kolthoff and Miller (*9*)
28 to 37	O_2			3*	Davies and Brink (*10*)
20	O_2			2.5*	Hill (*11*)
20 to 30	unspecified			2.5*	Höber (*12*)
20 to 25	unspecified			2.9*	Bull (*13*)
unspecified	unspecified			3*	Einstein diffusion equation (Höber, *12*)

* Values stated by authors but not experimentally determined. † Determined by dropping mercury electrode—mean value of six readings where drop time was 2 to 4 sec (see Kolthoff and Lingane, *14*). ‡ Diffusion current changed 4 percent per degree (*14*).

Table 2. Change in D with temperature calculated from values in Table 1

Temperature interval (°C)	Linear increase in D (%/°C rise in T)
16 to 18.2	2.8
16 to 22	3.1
16 to 25	3.0
16 to 25	4.3*
18.2 to 22	3.0
18.2 to 25	2.9
18.2 to 25	4.5*
22 to 25	2.4
22 to 25	5.7*

* The results based on Kolthoff and Miller's value $D = 2.6 \times 10^{-5}$ cm²/sec at 25°C are of doubtful significance. Kolthoff and Lingane (*14*) in their latest book have used the earlier experimental value of 2.38×10^{-5} cm²/sec at 25°C.

The decrease in the solubility of oxygen between 16° and 25°C is about 1.6 percent per degree. The relationship shown between the experimental determinations of four observers at four temperatures indicates an increase in D of about 3 percent per degree rise in temperature for oxygen within the same temperature range in water. Thus,

$$K_{T+1} = K_T \times \frac{103}{100} \times \frac{100}{101.6}.$$

That is,

$$K_{T+1} = K_T \times 1.014,$$

where K_T is the Krogh diffusion constant at temperature T.

Thus the increase in the Krogh diffusion constant is about 1 percent per degree rise in temperature within the range given.

4) Verduin has produced evidence of the misuse of the Krogh diffusion constant. Some physiologists have treated diffusion problems without confusion and it may be worth while recording some of them here for future reference. Hill (*11*) used both Krogh diffusion constants and true diffusivities, illustrating the interrelations of the two and their changes with temperature. Hill devoted some time to discussing the diffusivity of CO_2 in tissues (based on Krogh's value) which is still 1.2 times the diffusivity of O_2 when solubility differences are corrected. It is felt that the work of Hill has not received the attention it deserves; it has evidently escaped the notice of Prosser *et al* (*16*) and perhaps other physiologists. More recent biological studies of O_2 and CO_2 diffusion through tissues that clearly appreciate the nature of the diffusion coefficient include Briggs and Robertson (*17*), Roughton (*18*) and James (*19*).

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13 June 1955

Correction

The ultrasonic dosages given in W. J. Fry *et al.*, "Ultrasonic lesions in the mammalian central nervous system" [*Science* **122**, 517 (1955)] should be corrected as follows: For the lesion illustrated in Fig. 1, the dosage was 40 atm acoustic pressure amplitude and $3.9(10^2)$ cm/sec acoustic particle velocity amplitude. For the lesions illustrated in Figs. 2 and 3, the dosage was 41 atm acoustic pressure amplitude and $4.0(10^2)$ cm/sec acoustic particle velocity amplitude.

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Thomas Bradwardine His Tractatus de Proportionibus

I am sure both the author and the University of Wisconsin Press personnel welcomed the excellent review by Carl Boyer of H. Lamar Crosby, Jr.'s, recent volume on Thomas Bradwardine [*Science*, **122**, 562 (23 Sept. 1955)]. However, I believe one amendment to the review is in order. Boyer very graciously mentioned the work in medieval science being done at the University of Wisconsin, but in doing so he left the distinct impression that Crosby's volume was written here at Wisconsin in our department. As much as we would like to claim some part in the direction of the work that went into the writing of this volume, we must note that it was completed by Crosby under the stimulating guidance of Ernest Moody while the latter was at Columbia University. My only part in the volume was to recommend its consideration for publication by the University of Wisconsin Press and to add a foreword.

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24 October 1955

Separation of Complete and Incomplete Rh Antibodies by Centrifugation

The division of Rh antibodies into complete (saline agglutinins, bivalent) and incomplete (blocking, univalent) varieties is based on differences in their immunologic reactions. Physicochemical, electrophoretic, and biologic differences in these antibodies have been reviewed in recent publications from these laboratories, and additional data concerning their immunologic and electrophoretic nature have been presented (*1–3*). Although there are no experimental data dealing directly with the possibility of differences in molecular shape and size of Rh antibodies (*4*), Wiener has speculated that such differences probably exist (*5*). Previously, Deutsch *et al.* (*6*) and Pedersen (*7*), on the basis of ultracentrifuge studies of human serum, reported that β-isoagglutinins occurred to some extent in a "heavy" molecular weight fraction of serum globulin. In view of this work, an investigation was undertaken to determine whether Rh agglutinins could be separated from Rh incomplete antibodies by centrifugation. The present report presents the preliminary result of this investigation (*8*).

Serums were merely diluted with 1 vol of 1.0-percent sodium chloride solution, clarified by centrifugation at 4000 rev/min and then centrifuged for 7 hours at 40,000 rev/min in a Spinco No. 40 rotor with refrigeration. The first preparations consisted of four fractions representing four volumetric divisions of the 10-ml sample in each centrifuge tube. Fraction 1 consisted of the upper 3-ml portion, fraction 2 the next 3 ml, fraction 3 the next 3 ml and fraction 4 the remaining solution of about 1 ml plus the gelatinous pellet on the bottom.

The results of a typical fractionation in this preliminary series are shown in Table 1. The serum used in this run was prepared by mixing an incomplete anti-DE serum with a saline agglutinating anti-E serum in the ratio of 2.5 ml to 18.0 ml. The anti-DE serum was obtained from an O-Cde/cde individual who had been immunized to the Rh factor 13 years previously by a blood transfusion. Since then, there were repeated exposures to Rh antigen from pregnancies and more recently, following hysterectomy, from small injections of Rh positive blood as a volunteer donor. The serum had a titer in albumin of 1/4000 anti-D and 1/64 anti-E; there was no activity in saline. The anti-E saline agglutinating serum was obtained from an A-CDe/CDe individual immunized by cDE cells from blood transfusions and pregnancies. This serum's agglutinin titer was 1/512 in saline and 1/64 for incomplete antibodies, as estimated by the antiglobulin augmentation titer (*3*). The mixture of the two serums and the fractions produced by centrifugation resulted in titers shown in Table 1. Titrations were performed with O-CDe/cde, O-cdE/cde and B-cde/cde cells.

These preliminary results indicated that under the prescribed conditions of centrifugation, the saline agglutinating antibodies were sedimented more completely than were the incomplete antibodies. The saline agglutination reaction with the F-4 fraction was unusual in that the button of agglutinated cells could not be broken apart by the most vigorous shaking.

By repeated recycling of the F-4 fraction, and particularly the gelatinous pellet that separated out at the bottom of the centrifuge tube, it has been possible to separate the agglutinin from the incomplete antibody even more effectively.

Table 1. Antibody titers (expressed as dilutions of the original sample of serum) of serum fractions obtained by centrifugation.

Material	Protein concentration (%)	Agglutinins				
		Incomplete		Complete		
		anti-D	anti-E	anti-D	anti-E	anti-B
Serum	—	1/256	1/256	0	1/512	1/32
F-1	0.85	1/16	1/8	0	0	0
F-2	2.25	1/256	1/128	0	1/16	0
F-3	3.25	1/256	1/256	0	1/128	1/1
F-4	8.92	1/2000	±	0	1/4000	1/256